PCT/AU03/01365

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I, JULIE BILLINGSLEY, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. 2002952084 for a patent by THE UNIVERSITY OF QUEENSLAND as filed on 16 October 2002.

WITNESS my hand this
Fourth day of November 2003

J. Billingsley

JULIE BILLINGSLEY
TEAM LEADER EXAMINATION
SUPPORT AND SALES

AUSTRALIA

Patents Act 1990

PROVISIONAL SPECIFICATION

Applicants:

THE UNIVERSITY OF QUEENSLAND

Invention Title:

TREATMENT OF INFLAMMATORY BOWEL DISEASE

The invention is described in the following statement:

TREATMENT OF INFLAMMATORY BOWEL DISEASE

FIELD OF THE INVENTION

This invention relates to the treatment of inflammatory bowel disease with novel cyclic peptidic and peptidomimetic compounds which have the ability to modulate the activity of G protein-coupled receptors. The compounds preferably act as antagonists of the C5a receptor, and are active against C5a receptors on polymorphonuclear leukocytes and macrophages.

BACKGROUND OF THE INVENTION

All references, including any patents or patent applications, cited in this specification are hereby incorporated by reference. No admission is made that any reference constitutes prior art. The discussion of the references states what their authors assert, and the applicants reserve the right to challenge the accuracy and pertinency of the cited documents. It will be clearly understood that, although a number of prior art publications are referred to herein, this reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art, in Australia or in any other country.

G protein-coupled receptors are prevalent throughout the human body, comprising approximately 60% of known cellular receptor types, and mediate signal transduction across the cell membrane for a very wide range of endogenous ligands. They participate in a diverse array of physiological and pathophysiological processes, including, but not limited to those associated with cardiovascular, central and peripheral nervous system, reproductive, metabolic, digestive, immunological, inflammatory, and growth disorders, as well as other cell-regulatory and proliferative disorders. Agents which selectively modulate functions of G protein-coupled receptors have important therapeutic applications. These

receptors are becoming increasingly recognised as important drug targets, due to their crucial roles in signal transduction (G protein-coupled Receptors, IBC Biomedical Library Series, 1996).

One of the most intensively studied G protein-coupled receptors is the receptor for C5a. C5a is one of the most potent chemotactic agents known, and recruits neutrophils and macrophages to sites of injury, alters their morphology; induces degranulation; increases calcium mobilisation, vascular permeability (oedema) and neutrophil adhesiveness; contracts smooth muscle; stimulates release of inflammatory mediators, including histamine, TNF-α, IL-1, IL-6, IL-8, prostaglandins, and leukotrienes, and of lysosomal enzymes; promotes formation of oxygen radicals; and enhances antibody production (Gerard and Gerard, 1994).

Agents which limit the pro-inflammatory actions of C5a have potential for inhibiting chronic inflammation, and its accompanying pain and tissue damage. For these reasons, molecules which prevent C5a from binding to its receptors are useful for treating chronic inflammatory disorders driven by complement activation. Because such compounds act upstream from the various inflammatory mediators referred to above, and inhibit the formation of many of these compounds, they may have a more powerful effect in alleviating or preventing inflammatory symptoms.

In our previous applications No.PCT/AU98/00490, we described the three-dimensional structure of some analogues of the C-terminus of human C5a, and used this information to design novel compounds which bind to the human C5a receptor (C5aR), behaving as either agonists or antagonists of C5a. It had previously been thought that a putative antagonist might require both a C-terminal arginine and a C-terminal carboxylate for receptor binding and antagonist activity (Konteatis *et al*, 1994). We showed that in fact a terminal carboxylate group is not generally required either for high affinity binding to

C5aR or for antagonist activity. Instead we found that a hitherto unrecognised structural feature, a turn conformation, was the key recognition feature for high affinity binding to the human C5a receptor on neutrophils. As described in our Australian provisional application No. PR8334, filed on 17th October 2001, we used these findings to design constrained structural templates which enable hydrophobic groups to be assembled into a hydrophobic array for interaction with a C5a receptor. The entire disclosures of these specifications are incorporated herein by this reference.

Inflammatory bowel disease (IBD) is a group of serious, chronic relapsing inflammatory diseases affecting both the small and large intestine which remains relatively resistant to current treatments. Major forms of the disease are known, and Crohn's disease (regional bowel disease) and ulcerative colitis are the most common of these disorders. Because of the nature of their pathology, there are a number of autoimmune and immune-mediated diseases of the small and large bowel which are likely to benefit from treatment with these C5a antagonists. These include lymphocytic-plasmocytic enteritis, coeliac disease, collagenous colitis, lymphocytic colitis and eosinophilic enterocolitis. These conditions have been diagnosed in humans and in a number of animal species.

In 1999, approximately 1.7 million people in the United States alone were diagnosed with this debilitating disease. Satisfactory treatment of IBD is an unmet medical need, as existing therapeutic agents have not been successful in curtailing the disease and avoiding the need for surgery. Up to 40% of all ulcerative colitis patients undergo surgery, which typically includes either the removal of part of the large intestine or a full colostomy. While surgery is not curative for Crohn's disease, 75% of all patients will undergo at least one surgery in their lifetime, and up to 90% of these patients

require additional surgeries. A therapeutic agent which can successfully treat inflammatory bowel disease can enormously improve a patient's quality of life, while potentially saving the healthcare system millions of dollars in costs associated with invasive surgical procedures.

In these conditions, chronic inflammation, caused by a number of inflammatory mediators, has been implicated in pathogenesis. The complement system has been acknowledged as one of these inflammatory mediators, since increased levels of complement products are found in the colons of patients with IBD (Neilsen et al. 1996). Ulcerative colitis, also known as idiopathic colitis, is characterized by inflammation of the colon and rectum, which become inflamed and ulcerated; its cause is unclear, although antibodies to colonic epithelium and *E. coli* strain 0119 B14 are often present. Its severity varies, and the patients suffer frequent relapses. Crohn's disease, also called regional enteritis or regional ileitis, is characterized by inflammation, thickening and ulceration of the bowel wall, usually in the terminal part of the ileum, with oedematous mucosa or thickened soft tissue, mesenteric infiltration, thickened bowel wall, and often inflammatory masses, abscesses, or distended fluid-filled loops. Complications include fistulae, intramural sinus tracts, abscesses, perforations, toxic megacolon, obstruction of the bowel, or hydronephrosis, and there is an increased risk of adenocarcinoma in the ileum or colon.

In both these conditions extensive bed rest is often required, and in severe case the affected part of the bowel may be surgically removed, necessitating the use of an ostomy bag. The only therapeutic agents which are available are corticosteroids such as prednisolone, monoclonal antibodies directed against tumour necrosis factor, such as Remicade (infliximab), immunosuppressive agents such as methotrexate, azathioprine, cyclosporine, tacrolimus and mycophenolate mofetil, or other anti-

inflammatory agents such as sulphasalazine. These agents, especially the steroids, may be of limited effectiveness, and may have serious side effects. A variety of other agents, including corticosteroid derivatives such as budesonide antagonists and agonists of cytokines such as interleukin-10 and interleukin 11, and a nicotinic receptor agonist, are in various stages of clinical trial. However, there is a great need in the art for effective, non-toxic agents which do not require administration by injection, and which can be produced at reasonable cost. To our knowledge none of these approved or experimental agents, and in particular no small molecule agent, targets the C5a receptor.

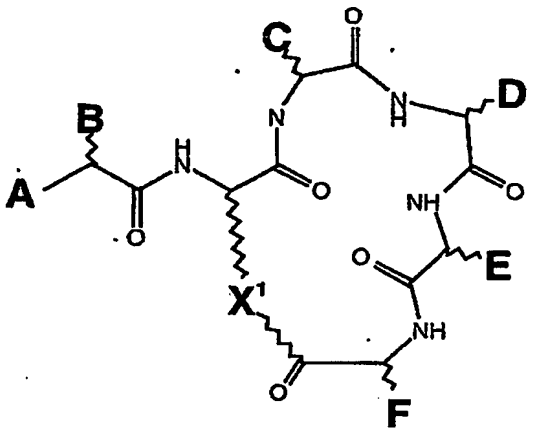
We now show for the first time that a specific inhibitor of the C5a receptor is able to ameliorate signs of damage in trinitrobenzenesulphonic acid (TNBS)-induced colitis in rats. This model has been used extensively to investigate the pathogenesis of IBD (Morris et al, 1989). This is the first reported case of an inhibitor of the complement system being used to modulate pathology in a model of IBD.

SUMMARY OF THE INVENTION

According to a first aspect, the invention provides a method of treatment of inflammatory bowel disease (IBD), comprising the step of administering an effective amount of an inhibitor of a G protein-coupled receptor to a subject in need of such treatment.

Preferably the a inhibitor is a compound which

(a) is an antagonist of a G protein-coupled receptor,
(b) has substantially no agonist activity, and
(c) is a cyclic peptide or peptidomimetic compound of formula I



where A is H, alkyl, aryl, NH_2, NH-alkyl, $N(alkyl)_2$, NH-aryl, NH-acyl, NH-benzoyl, $NHSO_3$, $NHSO_2$-alkyl, $NHSO_2$-aryl, OH, O-alkyl, or O-aryl;

B is an alkyl, aryl, phenyl, benzyl, naphthyl or indole group, or the side chain of a D- or L-amino acid such as L-phenylalanine or L-phenylglycine, but is not the side chain of glycine, D-phenylalanine, L-homophenylalanine, L-tryptophan, L-homotryptophan, L-tyrosine, or L-homotyrosine;

C is a small substituent, such as the side chain of a D-, L- or homo-amino acid such as glycine, alanine, leucine, valine, proline, hydroxyproline, or thioproline, but is preferably not a bulky substituent such as isoleucine, phenylalanine, or cyclohexylalanine;

D is the side chain of a neutral D-amino acid such as D-Leucine, D-homoleucine, D-cyclohexylalanine, D-homocyclohexylalanine, D-valine, D-norleucine, D-homo-norleucine, D-phenylalanine, D-tetrahydroisoquinoline, D-glutamine, D-glutamate, or D-tyrosine, but is preferably not a small substituent such as the side chain of glycine or D-alanine, a bulky planar side chain such as D-tryptophan, or a bulky charged side chain such as D-arginine or D-Lysine;

E is a bulky substituent, such as the side chain of an amino acid selected from the group consisting of L-

phenylalanine, L-tryptophan and L-homotryptophan, or is L-1-napthyl or L-3-benzothienyl alanine, but is not the side chain of D-tryptophan, L-N-methyltryptophan, L-homophenylalanine, L-2-naphthyl L-tetrahydroisoquinoline, L-cyclohexylalanine, D-leucine, L-fluorenylalanine, or L-histidine;

F is the side chain of L-arginine, L-homoarginine, L-citrulline, or L-canavanine, or a bioisostere thereof, ie. a side chain in which the terminal guanidine or urea group is retained, but the carbon backbone is replaced by a group which has different structure but is such that the side chain as a whole reacts with the target protein in the same way as the parent group; and

X is $-(CH_2)_nNH-$ or $(CH_2)_n-S-$, where n is an integer of from 1 to 4, preferably 2 or 3; $-(CH_2)_2O-$; $-(CH_2)_3O-$; $-(CH_2)_3-$; $-(CH_2)_4-$; $-CH_2COCHRNH-$; or $-CH_2-CHCOCHRNH-$, where R is the side chain of any common or uncommon amino acid.

In C, both the *cis* and *trans* forms of hydroxyproline and thioproline may be used.

Preferably A is an acetamide group, an aminomethyl group, or a substituted or unsubstituted sulphonamide group.

Preferably where A is a substituted sulphonamide, the substituent is an alkyl chain of 1 to 6, preferably 1 to 4 carbon atoms, or a phenyl or toluyl group.

In a particularly preferred embodiment, the compound has antagonist activity against C5aR, and has no C5a agonist activity.

The compound is preferably an antagonist of C5a receptors on human and mammalian cells including, but not limited to, human polymorphonuclear leukocytes and human macrophages. The compound preferably binds potently and selectively to C5a receptors, and more preferably has potent antagonist activity at sub-micromolar concentrations. Even more preferably the compound has a

receptor affinity IC50<25μM, and an antagonist potency IC50<1μM.

Most preferably the compound is compound **1**, compound **33**, compound **60** or compound **45** described in Provisional application no. PR8334.

The inhibitor may be used in conjunction with one or more other agents for the treatment of IBD, including but not limited to corticosteroids such as prednisolone and budesonide, other immunosuppressive agents such as infliximab (Remicade; Johnson & Johnson), or methotrexate or azathioprine, anti-inflammatory agents such as sulphasalazine, Colazal (balsalazide), and the like, and probiotics.

The compositions of the invention may be formulated for oral, parenteral, inhalational, intranasal, rectal or transdermal use, but oral or topical formulations are preferred. It is expected that most if not all compounds of the invention will be stable in the presence of metabolic enzymes, such as those of the gut, blood, lung or intracellular enzymes. Such stability can readily be tested by routine methods known to those skilled in the art.

Suitable formulations for administration by any desired route may be prepared by standard methods, for example by reference to well-known textbooks such as Remington: The Science and Practice of Pharmacy, Vol. II, 2000 (20th edition), A.R. Gennaro (ed), Williams & Wilkins, Pennsylvania.

While the invention is not in any way restricted to the treatment of any particular animal or species, it is particularly contemplated that the method of the invention will be useful in medical treatment of humans, and will also be useful in veterinary treatment, particularly of companion animals such as cats and dogs, livestock such as cattle, horses and sheep, and zoo animals, including non-human primates, large bovids, felids, ungulates and canids.

The compound may be administered at any suitable dose and by any suitable route. Oral, transdermal or intranasal administration is preferred, because of the greater convenience and acceptability of these routes. The effective dose will depend on the nature of the condition to be treated, and the age, weight, and underlying state of health of the individual treatment. This will be at the discretion of the attending physician or veterinarian. Suitable dosage levels may readily be determined by trial and error experimentation, using methods which are well known in the art.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the effects on a number of disease parameters of treatment of inflammatory bowel disease over a period of 24 hours in a rat model with PMX53, prednisolone, a combination of PMX53 and prednisolone, or infliximab.

Figure 2 shows the results of a study of the effects of the same treatments performed over 8 days.

Figure 3 compares the histological appearance of colonic sections from rats in the 8 day study.

DETAILED DESCRIPTION OF THE INVENTION

The invention will now be described by way of reference only to the following general methods and experimental examples.

For the purposes of this specification it will be clearly understood that the word "comprising" means "including but not limited to", and that the word "comprises" has a corresponding meaning.

As used herein, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "an enzyme" includes a plurality of such enzymes, and a reference to "an amino acid" is a reference to one or more amino acids. Unless defined otherwise, all technical and

scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any materials and methods similar or equivalent to those described herein can be used to practice or test the present invention, the preferred materials and methods are now described.

Abbreviations used herein are as follows:

Cit	citrulline
dCha	D-cyclohexylamine
DPhe	D-phenylalanine
IBD	inflammatory bowel disease
ip	intraperitoneal
iv	intravenous
LPS	lipopolysaccharide
PMN	polymorphonuclear granulocyte
PMSF	phenylmethylsulfonyl fluoride
sc	subcutaneous
TNBS	trinitrobenzenesulphonic acid

Throughout the specification conventional single-letter and three-letter codes are used to represent amino acids.

For the purposes of this specification, the term "alkyl" is to be taken to mean a straight, branched, or cyclic, substituted or unsubstituted alkyl chain of 1 to 6, preferably 1 to 4 carbons. Most preferably the alkyl group is a methyl group. The term "acyl" is to be taken to mean a substituted or unsubstituted acyl of 1 to 6, preferably 1 to 4 carbon atoms. Most preferably the acyl group is acetyl. The term "aryl" is to be understood to mean a substituted or unsubstituted homocyclic or heterocyclic aryl group, in which the ring preferably has 5 or 6 members.

A "common" amino acid is a L-amino acid selected from the group consisting of glycine, leucine, isoleucine, valine, alanine, phenylalanine, tyrosine, tryptophan, aspartate, asparagine, glutamate, glutamine, cysteine,

methionine, arginine, lysine, proline, serine, threonine and histidine.

An "uncommon" amino acid includes, but is not restricted to, D-amino acids, homo-amino acids, N-alkyl amino acids, dehydroamino acids, aromatic amino acids other than phenylalanine, tyrosine and tryptophan, ortho-, meta- or para-aminobenzoic acid, ornithine, citrulline, canavanine, norleucine, γ-glutamic acid, aminobutyric acid, L-fluorenylalanine, L-3-benzothienylalanine, and α,α-disubstituted amino acids.

Generally, the terms "treating", "treatment" and the like are used herein to mean affecting a subject, tissue or cell to obtain a desired pharmacological and/or physiological effect. The effect may be prophylactic in terms of completely or partially preventing a disease or sign or symptom thereof, and/or may be therapeutic in terms of a partial or complete cure of a disease.

"Treating" as used herein covers any treatment of, or prevention of disease in a vertebrate, a mammal, particularly a human, and includes: preventing the disease from occurring in a subject who may be predisposed to the disease, but has not yet been diagnosed as having it; inhibiting the disease, ie., arresting its development; or relieving or ameliorating the effects of the disease, ie., cause regression of the effects of the disease.

The invention includes the use of various pharmaceutical compositions useful for ameliorating disease. The pharmaceutical compositions according to one embodiment of the invention are prepared by bringing a compound of formula I, analogue, derivatives or salts thereof and one or more pharmaceutically-active agents or combinations of compound of formula I and one or more pharmaceutically-active agents into a form suitable for administration to a subject using carriers, excipients and additives or auxiliaries.

Frequently used carriers or auxiliaries include magnesium carbonate, titanium dioxide, lactose, mannitol

and other sugars, talc, milk protein, gelatin, starch, vitamins, cellulose and its derivatives, animal and vegetable oils, polyethylene glycols and solvents, such as sterile water, alcohols, glycerol and polyhydric alcohols. Intravenous vehicles include fluid and nutrient replenishers. Preservatives include antimicrobial, anti-oxidants, chelating agents and inert gases. Other pharmaceutically acceptable carriers include aqueous solutions, non-toxic excipients, including salts, preservatives, buffers and the like, as described, for instance, in Remington's Pharmaceutical Sciences, 20th ed. Williams & Wilkins (2000) and The British National Formulary 43rd ed. (British Medical Association and Royal Pharmaceutical Society of Great Britain, 2002; http://bnf.rhn.net), the contents of which are hereby incorporated by reference. The pH and exact concentration of the various components of the pharmaceutical composition are adjusted according to routine skills in the art. See Goodman and Gilman's The Pharmacological Basis for Therapeutics (7th ed., 1985).

The pharmaceutical compositions are preferably prepared and administered in dosage units. Solid dosage units include tablets, capsules and suppositories. For treatment of a subject, depending on activity of the compound, manner of administration, nature and severity of the disorder, age and body weight of the subject, different daily doses can be used. Under certain circumstances, however, higher or lower daily doses may be appropriate. The administration of the daily dose can be carried out both by single administration in the form of an individual dose unit or else several smaller dose units and also by multiple administration of subdivided doses at specific intervals.

The pharmaceutical compositions according to the invention may be administered locally or systemically in a therapeutically effective dose. Amounts effective for this use will, of course, depend on the severity of the

disease and the weight and general state of the subject. Typically, dosages used *in vitro* may provide useful guidance in the amounts useful for *in situ* administration of the pharmaceutical composition, and animal models may be used to determine effective dosages for treatment of the cytotoxic side effects. Various considerations are described, eg. in Langer, Science, 249: 1527, (1990). Formulations for oral use may be in the form of hard gelatin capsules, in which the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin. They may also be in the form of soft gelatin capsules, in which the active ingredient is mixed with water or an oil medium, such as peanut oil, liquid paraffin or olive oil.

Aqueous suspensions normally contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients may be suspending agents such as sodium carboxymethyl cellulose, methyl cellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents, which may be (a) a naturally occurring phosphatide such as lecithin; (b) a condensation product of an alkylene oxide with a fatty acid; for example, polyoxyethylene stearate; (c) a condensation product of ethylene oxide with a long chain aliphatic alcohol, for example, heptadecaethylenoxycetanol; (d) a condensation product of ethylene oxide with a partial ester derived from a fatty acid and hexitol such as polyoxyethylene sorbitol monooleate, or (e) a condensation product of ethylene oxide with a partial ester derived from fatty acids and hexitol anhydrides, for example polyoxyethylene sorbitan monooleate.

The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to known methods using suitable dispersing or wetting

agents and suspending agents such as those mentioned above. The sterile injectable preparation may also a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents which may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed, including synthetic mono-or diglycerides. In addition, fatty acids such as oleic acid may be used in the preparation of injectables.

Compounds of formula I may also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles, and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine, or phosphatidylcholines.

Dosage levels of the compound of formula I of the present invention will usually be of the order of about 0.5mg to about 20mg per kilogram body weight, with a preferred dosage range between about 0.5mg to about 10mg per kilogram body weight per day (from about 0.5g to about 3g per patient per day). The amount of active ingredient which may be combined with the carrier materials to produce a single dosage will vary, depending upon the host to be treated and the particular mode of administration. For example, a formulation intended for oral administration to humans may contain about 5mg to 1g of an active compound with an appropriate and convenient amount of carrier material, which may vary from about 5 to 95 percent of the total composition. Dosage unit forms will generally contain between from about 5mg to 500mg of active ingredient.

It will be understood, however, that the specific dose level for any particular patient will depend upon a

variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

In addition, some of the compounds of the invention may form solvates with water or common organic solvents. Such solvates are encompassed within the scope of the invention.

The compounds of the invention may additionally be combined with other therapeutic compounds to provide an operative combination. It is intended to include any chemically compatible combination of pharmaceutically-active agents, as long as the combination does not eliminate the activity of the compound of formula I of this invention.

General Methods

Cyclic peptide compounds of formula I are prepared according to methods described in detail in our earlier applications No. PCT/AU98/00490 and PR8334, the entire disclosures of which are incorporated herein by this reference. While the invention is specifically illustrated with reference to the compound AcF-[OPdChaWR] (PMX53), whose corresponding linear peptide is Ac-Phe-Orn-Pro-dCha-Trp-Arg, it will be clearly understood that the invention is not limited to this compound.

Compounds **1-6**, **17**, **20**, **28**, **30**, **31**, **36** and **44** disclosed in International patent application No.PCT/AU98/00490 and compounds **10-12**, **14**, **15**, **25**, **33**, **35**, **40**, **45**, **48**, **52**, **58**, **60**, **66**, and **68-70** disclosed for the first time in Australian provisional application No. PR8334 have appreciable antagonist potency (IC50 < 1 μM) against the C5a receptor on human neutrophils. PMX53 and compounds **33**, **45** and **60** of PR8334 are most preferred.

We have found that all of the compounds of

formula I which have so far been tested have broadly similar pharmacological activities, although the physicochemical properties, potency, and bioavailability of the individual compounds varies somewhat, depending on the specific substituents.

The following general tests may be used for initial screening of candidate inhibitor of G protein-coupled receptors, and especially of C5a receptors.

Receptor-Binding Assay

Assays are performed with fresh human PMNs, isolated as previously described (Sanderson *et al*, 1995), using a buffer of 50 mM HEPES, 1 mM $CaCl_2$, 5 mM $MgCl_2$, 0.5% bovine serum albumin, 0.1% bacitracin and 100 µM phenylmethylsulfonyl fluoride (PMSF). In assays performed at 4°C, buffer, unlabelled human recombinant C5a (Sigma) or peptide, Hunter/Bolton labelled ^{125}I-C5a (~ 20 pM) (New England Nuclear, MA) and PMNs (0.2 x 10^6) are added sequentially to a Millipore Multiscreen assay plate (HV 0.45) having a final volume of 200 µL/well. After incubation for 60 min at 4°C, the samples are filtered and the plate washed once with buffer. Filters are dried, punched and counted in an LKB gamma counter. Non-specific binding is assessed by the inclusion of 1mM peptide or 100 nM C5a, which typically results in 10-15% total binding.

Data are analysed using non-linear regression and statistics with Dunnett post-test.

Myeloperoxidase Release Assay for Antagonist Activity

Cells are isolated as previously described (Sanderson *et al*, 1995) and incubated with cytochalasin B (5µg/mL, 15 min, 37°C). Hank's Balanced Salt solution containing 0.15% gelatin and peptide is added on to a 96 well plate (total volume 100 µL/well), followed by 25 µL cells (4x10^6/mL). To assess the capacity of each peptide to antagonise C5a, cells are incubated for 5 min at 37°C with each peptide, followed by addition of C5a (100

nM) and further incubation for 5 min. Then 50 µL of sodium phosphate (0.1M, pH 6.8) is added to each well, the plate was cooled to room temperature, and 25 µL of a fresh mixture of equal volumes of dimethoxybenzidine (5.7 mg/mL) and H_2O_2 (0.51%) is added to each well. The reaction is stopped at 10 min by addition of 2% sodium azide. Absorbances are measured at 450 nm in a Bioscan 450 plate reader, corrected for control values (no peptide), and analysed by non-linear regression.

Example 1: Effect of PMX53 on inflammatory bowel disease

PMX53 (AcF-[OpdChaWR]) was tested for its ability to ameliorate signs of damage in trinitrobenzenesulphonic acid (TNBS)-induced colitis in rats. This model has been used extensively to investigate the pathogenesis of IBD (Morris et al, 1989).

Male Wistar rats (250-300g) were starved for 24 hours prior to being anaesthetised with ketamine (80 mg/kg i.p.) and xylazine (8 mg/kg i.p.). A 1.7 mm outer diameter polyethylene catheter was then inserted intracolonically to a distance of 8cm from the anus. 120mg/kg TNBS (50mg/mL solution) along with 250µL Ethanol (100%) was then instilled to rats, which remained in the head-down position for 30 min to prevent leakage. Sham-operated rats (rats without colitis) received either saline alone or 250µL ethanol and saline. Rats were then allowed to recover under observation.

Two time-frames were chosen for this study, an acute time frame(24 hours), and a chronic time frame (8-days). During the course of the study both body weight and food eaten were measured. In the 24 hour study, PMX53-, prednisolone- and combination-treated rats were treated daily, starting 2-days prior to instillation of TNBS (prevention). Rats treated with the TNF-α antibody, infliximab, were dosed intravenously on one occasion, 2

days prior to TNBS instillation. The following treatment groups were used in this 24 hour model:

(a) PMX53 (10mg/kg, in olive oil, oral),

(b) PMX53 (0.3mg/kg, in 30% polyethylene glycol, subcutaneous),

(c) prednisolone (1mg/kg, in 80% polyethylene glycol, subcutaneous),

(d) a combination of PMX53 (0.3mg/kg, in 30% polyethylene glycol, subcutaneous), and prednisolone (1mg/kg, in 80% polyethylene glycol, subcutaneous), and

(e) infliximab (1mg/kg, in saline, intravenous).

In the 8-day study, drug-treated rats were treated both prior to colitis induction (2 days before; prevention) or following colitis induction (24 hours after; reversal). The following treatment groups were used in this 8-day study:

(a) PMX53 (10mg/kg, in olive oil; oral, both pre- and post-induction),

(b) PMX53 (0.3mg/kg, in 30% polyethylene glycol, subcutaneous, pre-induction only),

(c) prednisolone (1mg/kg, in 80% polyethylene glycol, subcutaneous, both pre- and post-induction),

(d) a combination of PMX53 (10mg/kg, in olive oil, oral) and prednisolone (1mg/kg, in 80% polyethylene glycol, subcutaneous) (pre-induction only), and

(e) infliximab (1mg/kg, in saline, intravenous, pre-induction only).

Following the completion of the study, rats were anaesthetised with ketamine (80 mg/kg i.p.) and xylazine (12 mg/kg i.p.) and the distal 8cm colon removed, cut open and cleaned with 10 mL saline. Colons were then macroscopically scored under blind conditions on a scale of 1-14, as follows:

Ulceration	Diarrhea	Adhesions
0- No damage	0- Absent	0- Absent
1- Focal hyperemia	1- Mild	1- Mild
2- Hyperemia and bowel thickening	2- Severe	2- Severe
3- Ulceration at 1 site		
4- Ulceration at 2 sites		
5- Ulceration >1cm		
6-10- Ulceration >2cm; increase score by 1 for each additional cm.		

Sections of the colon were then removed, and stored in formaldehyde solution for histopathological analysis. Separate sections (0.2-0.4 gm) were also removed and homogenized in 1mL phosphate-buffered saline, for myeloperoxidase determination, as a measure of neutrophil accumulation, and measurement of TNF-α levels. The remaining sections of colon were weighed and placed in an 80°C oven overnight for wet-to-dry weight determination, as a measure of edema.

The results for a number of parameters are summarized in Figure 1.

In the 24-hour study, PMX53-treated rats had significantly lower colon macroscopic scores and higher body weights and food intake. The level of colon edema, neutrophil accumulation and colon TNF-α levels were also significantly reduced. In comparison to prednisolone and infliximab, which are currently used in therapy for IBD, PMX53-treated rats displayed a greater inhibition of the parameters measured. These results show that blockade of the inflammatory protein C5a by PMX53 significantly improves disease pathology in the acute 24-hour model of IBD, to an extent at least as great as that achieved by prednisolone or infliximab.

In the 8-day study, PMX53 pre-treated rats as well as post-treated rats lost significantly less body

weight and ate significantly more food compared to colitis control animals. These rats also had significantly lower colon macroscopic scores, colon edema, neutrophil accumulation and colon TNF-α levels. Most importantly, PMX53-treated rats also had reduced mortality compared to colitis control rats. In comparison, only prednisolone pre-treated rats displayed any improvement in the disease parameters measured. Infliximab-treated rats also had significantly reduced disease parameters, however to a lesser extent than those observed in PMX53-treated rats.

Histologically, colonic sections of rats treated with PMX53 displayed a decrease in inflammatory cell accumulation and haemorrhage formation, and this is shown in Figure 3. These results surprisingly show that blockade of the inflammatory protein C5a by PMX53 both prevents and reverses disease pathology in a rat model of chronic colitis. This effect of reversal of pathology was not seen in rats treated with prednisolone.

In summary, these studies demonstrate for the first time that an inhibitor of the complement system has beneficial effects in an established model of IBD. The improvements seen with PMX53 were greater than those seen with prednisolone or infliximab. This indicates that PMX53 will be useful in the clinical treatment of IBD.

Example 2: Assessment of clinical efficacy

The clinical efficacy in humans of compounds found to be effective in animal models may be determined using standard clinical trial methods.

For example, in a randomized, placebo-controlled, phase II trial in the treatment of patients with mild-to-moderate Crohn disease will typically employ at least two dose levels of the test compound. A decrease of greater than 70 points on the Crohn's Disease Activity Index (CDAI), a standardized tool designed to measure disease activity specifically, is a primary endpoint for indication of efficacy. Disease remission is a secondary

endpoint. A treatment effect advantage is established if significantly more recipients of the test compound than placebo recipients are found to have CDAI scores consistent with symptom resolution during an acute exacerbation of the inflammatory bowel disease.

For ulcerative colitis, the effects of treatment with the test compound are assessed in patients with symptoms of active ulcerative colitis who have either not previously been treated, or who are also receiving standard medical treatment. The endpoints are induction of complete remission or significant improvement in signs and symptoms of ulcerative colitis, as reflected in changes in a colitis activity index (CAI) score. The CAI score assesses stool frequency, rectal bleeding, endoscopic appearance of the colon, and includes a physician's global assessment.

For each condition the adverse effect and tolerability profiles of the test compound are also monitored.

DISCUSSION

Cyclic peptides have several important advantages over acyclic peptides as drug candidates (Fairlie et al 1995, Fairlie et al, 1998, Tyndall and Fairlie, 2001). The cyclic compounds described in this specification are stable to proteolytic degradation for at least several hours at 37°C in human blood or plasma, in human or rat gastric juices, or in the presence of digestive enzymes such as pepsin, trypsin and chymotrypsin. In contrast, short linear peptides composed of L-amino acids are rapidly degraded to their component amino acids within a few minutes under these conditions. A second advantage lies in the constrained single conformations adopted by the cyclic and non-peptidic molecules, in contrast to acyclic or linear peptides, which are flexible enough to adopt multiple structures in solution other than the one required for receptor-binding. Thirdly, cyclic compounds

such as those described in this invention are usually more lipid-soluble and more pharmacologically bioavailable as drugs than acyclic peptides, which can rarely be administered orally. Fourthly, the plasma half-lives of cyclic molecules are usually longer than those of peptides.

It will be apparent to the person skilled in the art that while the invention has been described in some detail for the purposes of clarity and understanding, various modifications and alterations to the embodiments and methods described herein may be made without departing from the scope of the inventive concept disclosed in this specification.

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Figure 1: 24-Hour Study

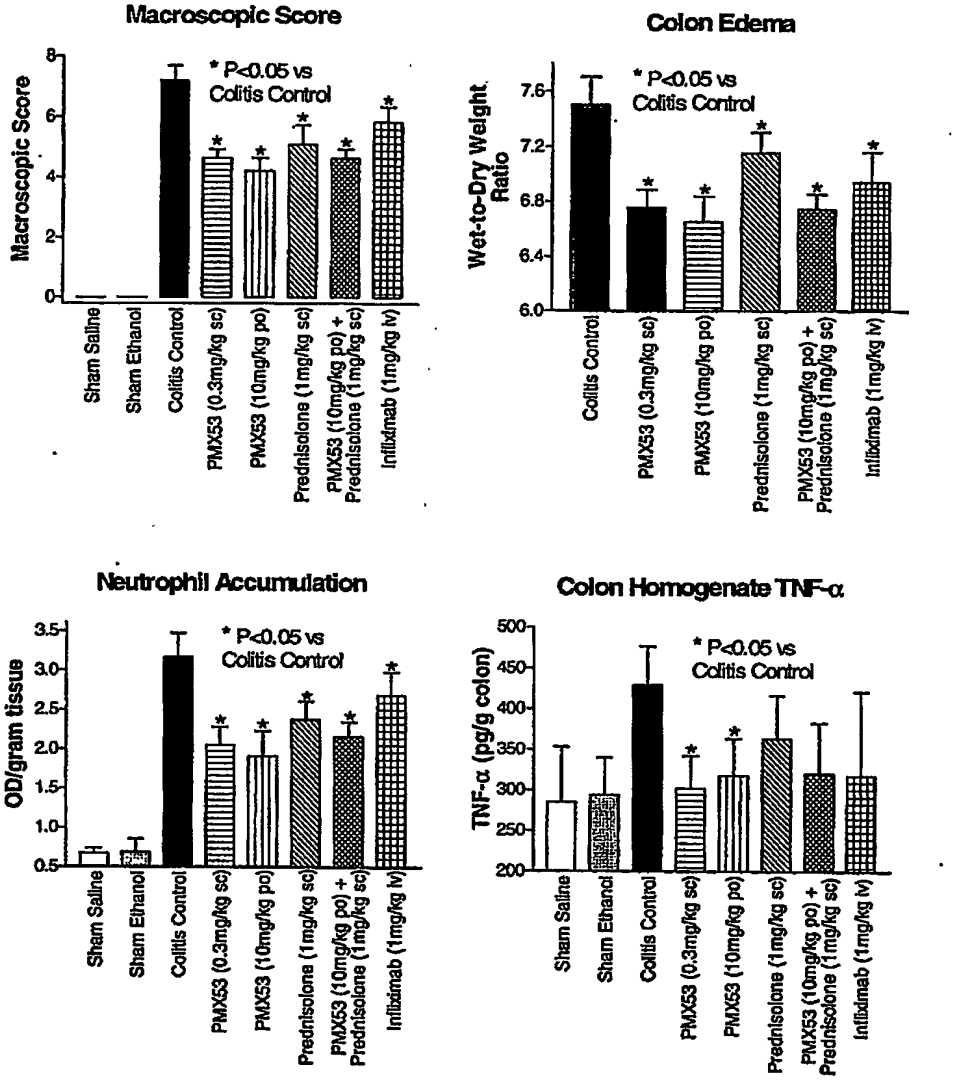

Figure 2: 8-Day Study

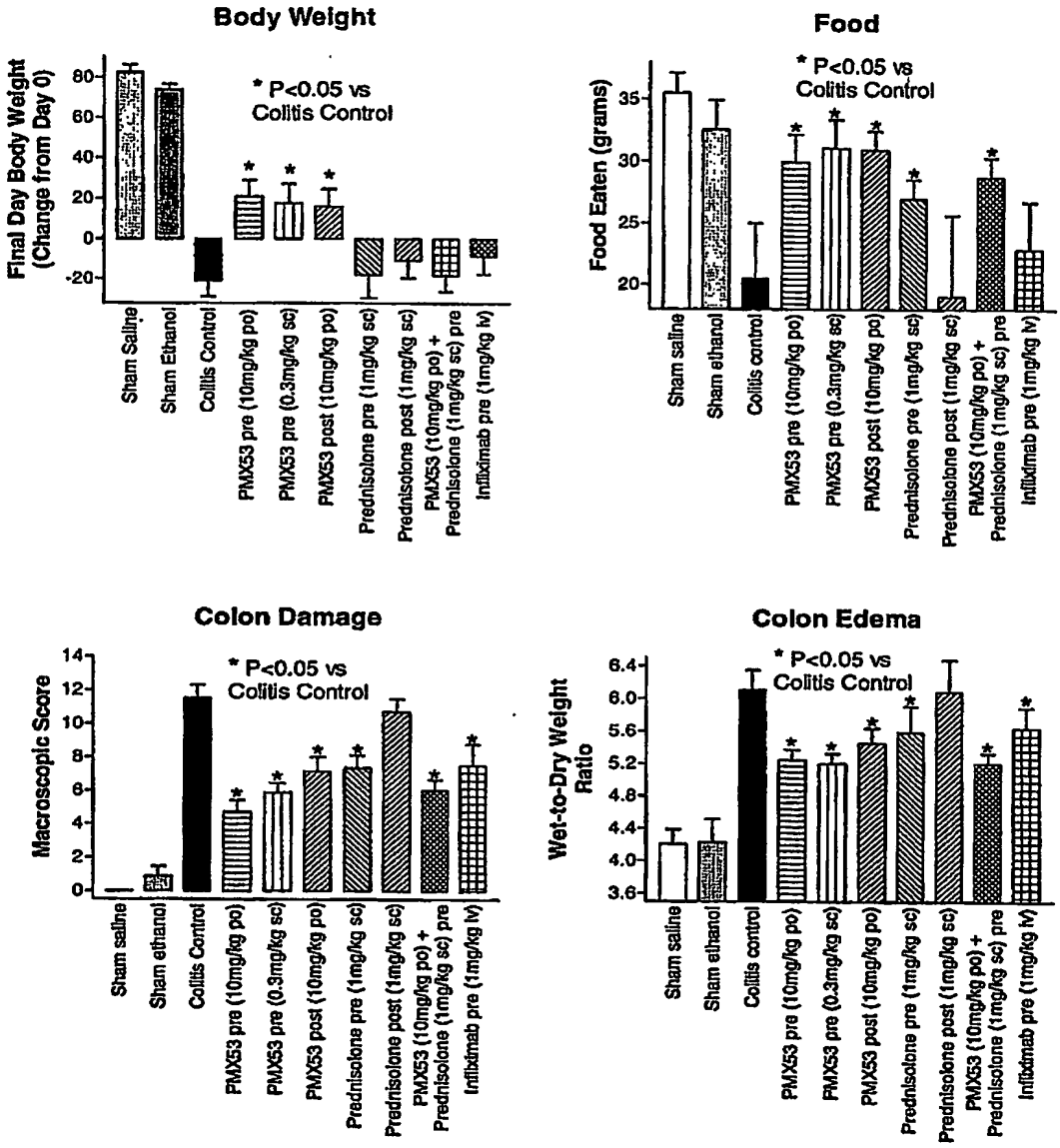

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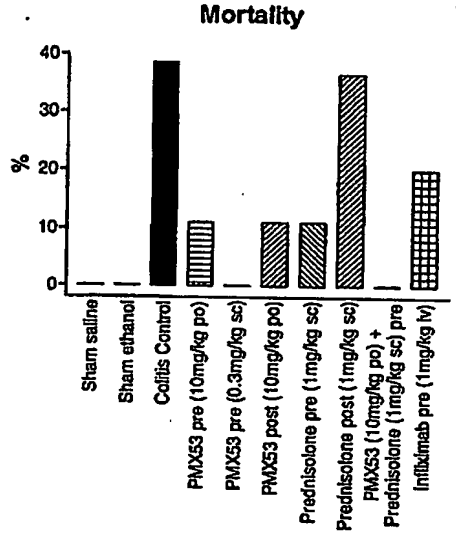
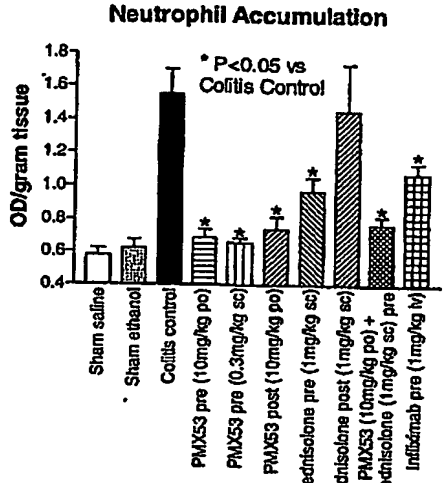
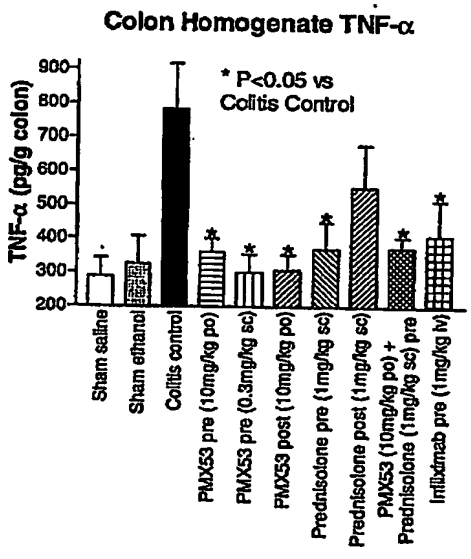
Figure 2 continued







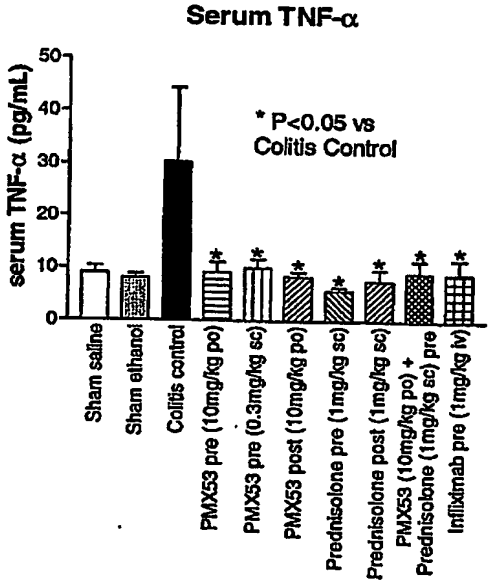
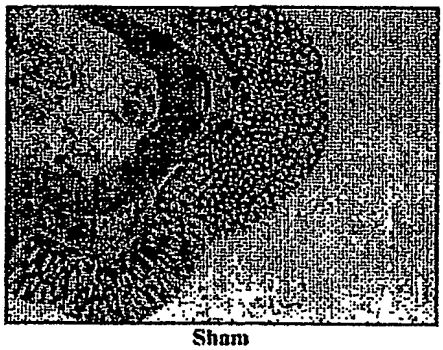
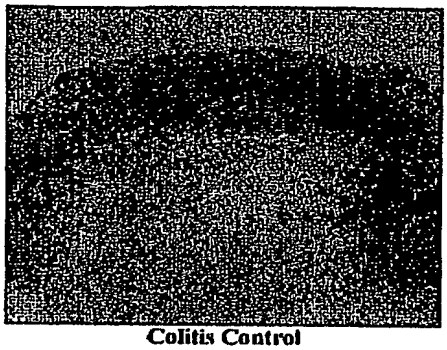


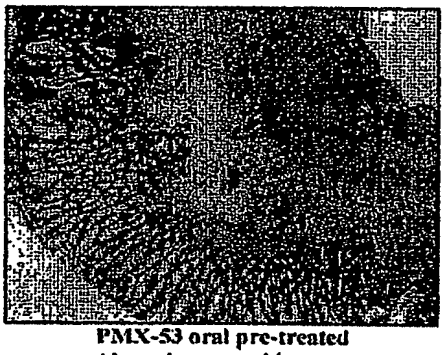
Figure 3



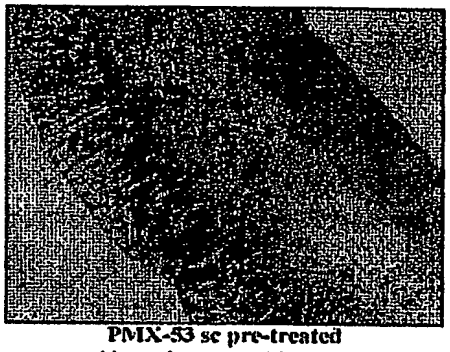
Sham
Normal crypt architecture
and muscle layers



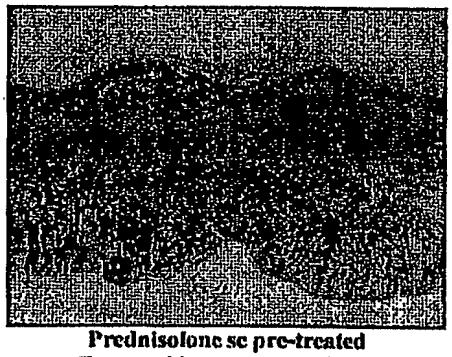
Colitis Control
Complete loss of crypt architecture
Muscle edema and inflammation



PMX-53 oral pre-treated
Normal crypt architecture
Muscle edema without inflammation



PMX-53 sc pre-treated
Normal crypt architecture
Muscle edema and inflammation



Prednisolone sc pre-treated
Crypt architecture mostly intact
Muscle edema without inflammation

Prednisolone sc post-treated
Crypt architecture mostly necrosed
Muscle inflammation, no edema